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Practical Convergent Laboratory-Scale Synthesis of a CCR5 Receptor Antagonist

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Supporting Information

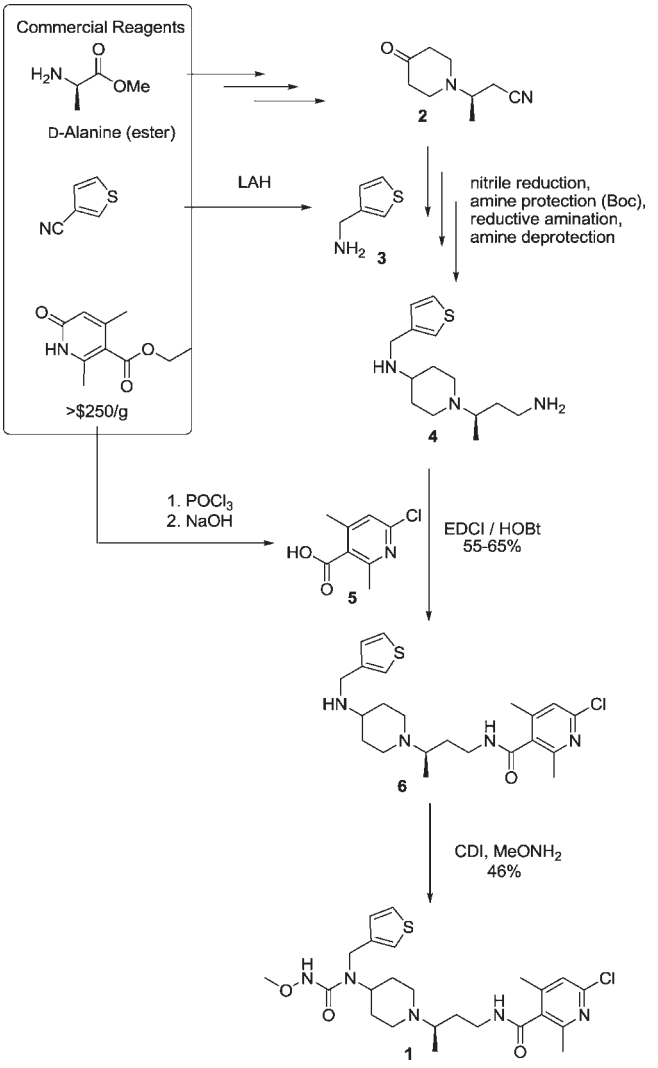
ABSTRACT: An efficient laboratory-scale synthesis has been developed for the selective CCR5 antagonist **1**. The convergent route has a longest linear sequence of nine steps (15 steps overall), and has overall yields of 18−25%. The route has enabled the preparation of 550 g of **1**.

INTRODUCTION

The human immunodeficiency virus (HIV) gains entry to human white blood cells via a receptor-mediated process involving one of two human chemokine coreceptors, CXCR4 or CCR5. The receptors are 7-transmembrane G-protein-coupled receptors (GPCR) and are expressed on the surface of a variety of cells, including CD4+ T cells. Inhibition of the viral interaction with the chemokine receptor, through the use of specific antagonists, has been clinically validated as a therapeutic approach to treat HIV infection for both CCR5[1] and CXCR4[2,3] antagonists. We have previously described the discovery and development of the CXCR4 chemokine receptor antagonist AMD070,[4,5] an oral agent that entered clinical development for the treatment of T-(CXCR4)-tropic HIV-1 infected patients.[3] In addition we have recently disclosed our efforts to indentify novel CCR5 antagonists.[6] Similarly, the discovery and development of a number of CCR5 antagonists, including maraviroc (Selzentry),[7] vicriviroc,[8] TAK-779,[9] and the experimental drug R05114436,[10] have been described. Herein, we describe the practical laboratory-scale synthesis of a novel selective CCR5 antagonist AMD15548 (**1**).[11]

The medicinal chemistry route to **1** is shown in Scheme 1, and was the starting point for process development. The single chiral centre originated from D-alanine (methyl ester), and was incorporated into the piperidone synthon (**2**) over several steps (discussed below and in Scheme 2). In the initial medicinal chemistry routes, the nitrile was reduced and the primary amine protected (BOC). Commercial 3-cyanothiophene was reduced with lithium aluminum hydride (LAH) to provide 3-aminomethyl thiophene (**3**), which was coupled with **2** via standard reductive amination conditions to provide **4** (after amine deprotection). The substituted nicotinic acid derivative **5** was synthesized in a two step chlorination/hydrolysis from the costly commercial pyridone. A peptide coupling using 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carboxdiimide (EDCI) with **4** provided amide **5**, and the substituted urea was installed via reaction with 1,1′-carbonyldiimidazole (CDI) and methoxylamine to give **1**.

Scheme 1. Medicinal Chemistry Synthesis of 1



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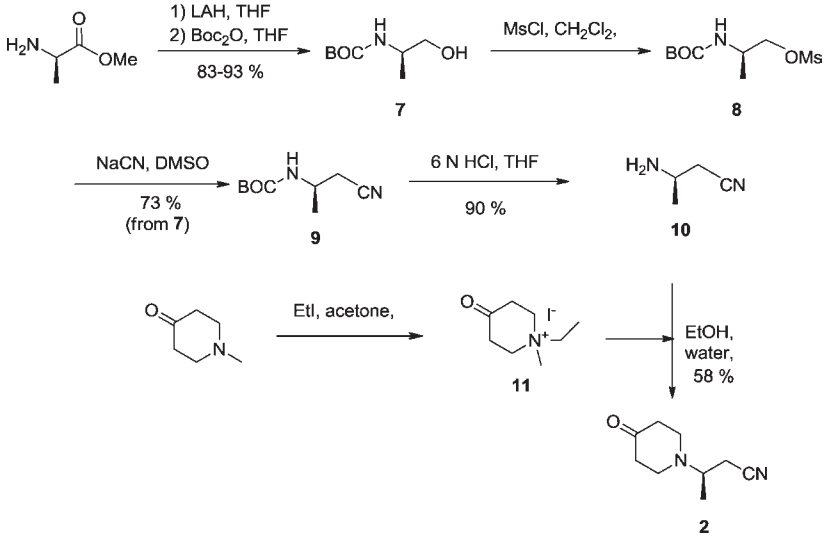
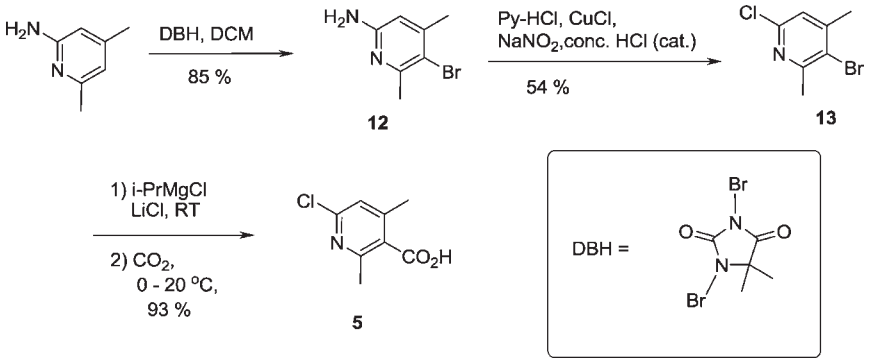
Scheme 2. Synthesis of ketone intermediate 2



Scheme 3. Scale-up synthesis of 5



RESULTS AND DISCUSSION

Preparation of Piperdone 2. We briefly investigated the possibility of preparing **2** via an asymmetric conjugate addition between a protected piperidone and crotononitrile,[12] but abandoned the strategy due to lack of initial success with establishing enantioselectivity, coupled with the limited practical availability of isomerically pure crotononitrile. Following the Med Chem route of chemical reduction of commercial D-alanine methyl ester, the reaction could be carried out at 20-L scale via addition of the ester to a solution of lithium aluminum hydride in THF at <20 °C (Scheme 2). A Fieser-type workup[13] was employed—the hydrated aluminum salts formed granular white solids which were easily held in suspension. A solution of di-*tert*-butyl dicarbonate was added directly to the reaction mixture, and the aluminum salts were removed by filtration to give the product **7** in 83−93% yields as a solid. The conversion to mesylate **8** was straightforward and typically was used immediately to form the nitrile with sodium cyanide at ∼50 °C. At the completion of the reaction, filtration through silica gel removed any polar impurities and/or salts, and the amine **10** was liberated via treatment with aqueous acid. Coupling of amine **10** with the commercially available piperidone salt **11** occurred via a Hofmann elimination/conjugate addition sequence[14] under mild conditions to form **2** directly. Purification did require silica gel chromatography to produce **2** in >99% chemical purity and ee. Yields of **2** from commercial D-alanine methyl ester were typically in the 35% range for 300−400 g batches.

Preparation of Nicotinic Acid 5. The medicinal chemistry route to **5** was only capable of supplying single gram amounts as the commercial pyridone reagent was cost prohibitive (>$250/g) and lead times were typically greater than 6 weeks. As an alternative, based on availability and cost, 2-amino-4,6-dimethylpyridine was selected as a reagent, and a route was developed (Scheme 3). Rather than using the typical *N*-bromosuccinimide (NBS) to brominate the pyridine, we instead employed 5,5-dimethyl-1,3-dibromohydantoin[15] (DBH). DBH gave a cleaner, more regioselective bromination to give **12**. With NBS, small amounts of 3,5-dibrominated material (1−5%), as well as some bromination of the methyl groups, could be detected by ^{1}H NMR.[16] Once the reaction was complete, polar impurities were removed via a filtration through silica gel, and **12** was isolated via crystallization.

The Sandmeyer conversion of **12** to **13**[17] used pyridine-HCl/catalytic concentrated HCl rather than stoichiometric amounts

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of HCl. A catalytic amount of HCl was added to the reaction mixture after all other reagents were added, and it provided a cleaner overall reaction profile, a faster and more predictable reaction rate, and higher and more reproducible yields. For this reaction, a range of 0−5% v/w concentrated HCl (relative to **12**) was investigated, with 2% being optimal (i.e. complete predictable reaction in 1.5 h). Silica gel filtration was used to remove polar impurities, and the product **13** was isolated as a crystalline solid in 54−60% yields following trituration. The regioselective halogen/metal exchange, originally carried out on small scale at −78 °C with *n*-BuLi, could be instead carried out on scale at 0 °C using the Grignard reagent isopropylmagnesium chloride and LiCl to regioselectively obtain the acid **5**, after quenching with CO_2. The noncryogenic conditions were reliable in our hands and provided **5** in 400-g batches as a crystalline solid isolated from water.

Preparation of Synthons 3 and 14. Commercial 3-cyanothiophene was readily reduced with LAH in THF to provide the amine **3**. The Fieser workup gave granular solids which could be easily removed by filtration, and **3** could be isolated in >90% GC purity through fractional vacuum distillation in 85−92% yields (Scheme 4).

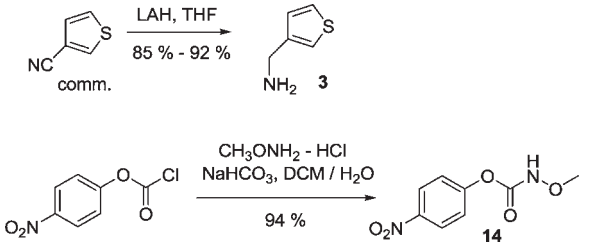
Similarly, a single transformation from *p*-nitrophenyl chloroformate and methoxylamine HCl provided **14**, which was isolable as a crystalline solid via filtration directly from the reaction mixture. In general, batches of **14** were produced within a week of being required for subsequent coupling reactions.

Assembly of Synthons to form 1. With all three synthons in hand, the convergent assembly of **1** was achieved as shown in Scheme 5. A reductive amination between aminomethyl thiophene **3** and ketone **2** provided the amine **15** in 82−90% yields at 500-g scale. During the workup, the product **15** was initially driven into the aqueous layer (at pH ≈ 4) via addition of acid, and nonbasic impurities were efficiently removed in the organic layer. A subsequent pH swing to 8 enabled the extraction of **15** into dichloromethane. Crystallization of the residue from hexanes and MTBE enabled isolation of **15** in >98% purity. The reduction of the nitrile was carried out with solid LAH in MTBE, which provided amine **4**, effectively eliminating the need for an intermediate BOC protection (as was used in the Med Chem route). The amine **4** was coupled, without further purification, with the acid **5** using EDCI and HOBt.[11] As was the case with **15**, a pH swing was used to remove nonbasic impurities. Following extraction and concentration, the product **6** could be isolated via MTBE crystallization in >98% purity.

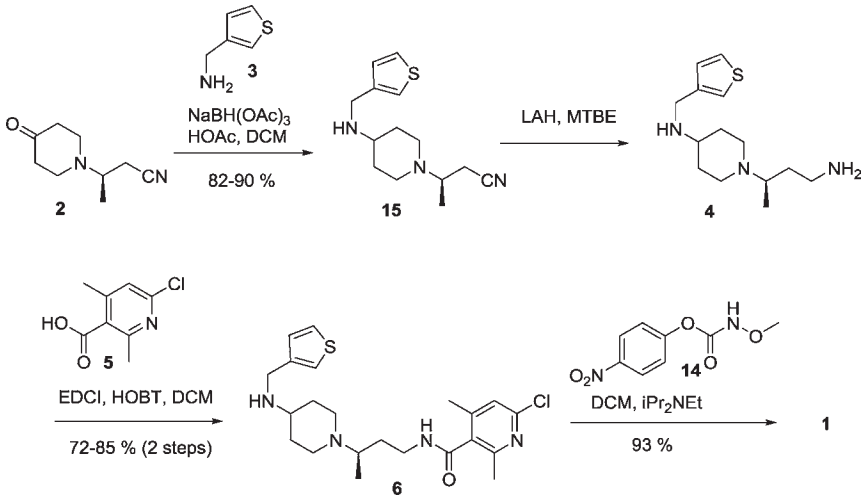
The incorporation of the urea functionality was accomplished initially by using carbonyldiimidazole (with methoxylamine).[11] The coupling yields were variable, however, and chromatographic purification was required. An improvement was the reaction of the amine **6** with the carbamate reagent **14**. After addition of water, and acidification to pH ∼1.5, the *p*-nitrophenol side product was removed by a wash with ether. The product **1** was then isolated from the aqueous layer with dichloromethane at pH 8. A silica gel plug filtration permitted the pure product **1** to be isolated as an amorphous freebase solid upon concentration.

Overall, these routes were used to provide a single batch of 550 g of **1** for toxicological studies. It should be noted that the procedures contained herein represent an initial streamlining of the process with the goals of decreasing costs, removing cryogenic conditions, decreasing the number of chromatographic steps, and providing a basis for further scale-up efforts. Significant further development would be required to enable a safe and practical multikilogram process, including: calorimetric hazard analysis, replacement of some process solvents (MTBE for ether and heptanes for hexanes, for example), removal or replacement of chemical desiccants, further isolation of crystalline solids from process streams (instead of concentration to dryness) and further reduction or removal of chromatographic steps. Further development was precluded by an organizational restructure.

Scheme 4. Preparation of Remaining Synthons



Scheme 5. Assembly of 1



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CONCLUSIONS

An efficient convergent laboratory-scale process to synthesize the selective CCR5 antagonist **1** has been developed which avoids cryogenic conditions and minimizes column purification steps (two silica gel plug filtrations and two columns). The sequence uses only one protecting group (Boc protection of amine **7**) and is thus efficient from an atom-economy perspective. The convergent route has a longest linear sequence of nine steps (15 steps overall) and is based on strategic assembly around a piperidone core (**2**), in which the aminothiophene **3**, nicotinic acid **5**, and carbamate reagent **14** are incorporated sequentially. Overall, the yields on scale are typically 18−25%, and the route has been carried out to provide 550 g of **1**.

EXPERIMENTAL SECTION

^{1}H NMR (300 MHz) and ^{13}C (75 MHz) spectra were recorded using $CDCl_3$ solvent with TMS as an internal standard (unless otherwise noted). All reactions were conducted under an inert (nitrogen) atmosphere, unless stated otherwise. HPLC was performed using reverse-phase conditions with a C18 column and acetonitrile/water mobile phase, and UV detection (220 or 254 nm).

Preparation of (*R*)-(2-Hydroxy-1-methyl-ethyl)-carbamic Acid *tert*-Butyl Ester (7). A 1.0 M solution of lithium aluminum hydride in THF (3.9 L, 3.9 mol) was cooled to 4 °C under argon (typically <5 °C). D-Alanine methyl ester HCl (376.77 g, 2.79 mol) was added in portions (~25 g/portion) over 90 min at 5−20 °C. The addition funnel and walls of the reactor were rinsed with THF (150 mL). The reaction was warmed over 20 min from 3.8 to 15.8 °C. The reaction was cooled to 13.3 °C (10−15 °C target) and quenched with water (148 mL), over 60 min, maintaining a temperature below 22 °C. A 4 N sodium hydroxide solution (226 mL) was added over 10 min, followed by water (450 mL) over 5 min. A solution of di-*tert*-butyl dicarbonate (534 g, 2.42 mol) in dry THF (1.2 L) was added to the suspension, and stirring continued for an additional 18 h at 22 °C. The suspension was filtered through a glass frit, and the collected solids were washed with ethyl acetate (6.5 L). The resulting filtrate was concentrated in vacuo and taken up in dichloromethane (1 L), dried over sodium sulfate, and reconcentrated to afford 453 g at 96.9 wt % (NMR estimate of solvent content: corrected yield 439 g, 93 %) of **7** as an orange solid. ^{1}H NMR 4.64 (bs, 1H), 3.74.3.79 (m, 1H), 3.61−3.66 (m, 1H), 3.49−3.54 (m, 1H), 2.67 (bs, 1H), 1.45 (s, 9H), 1.14 (d, J = 6.9 Hz, 3H).

Synthesis of (*R*)-Methanesulfonic Acid 2-*tert*-Butoxycarbonylaminopropyl Ester (8). To a solution of (*R*)-(2-hydroxy-1-methyl−ethyl)-carbamic acid *tert*-butyl ester **7** (828.96 g, 4.73 mol) in dry dichloromethane (9.1 L) was added triethylamine (1.155 L, 8.29 mol). The mixture was cooled (5.4 °C) and stirred for 1 h. To the mixture above was added methane sulfonyl chloride (400 mL, 5.2 mol) in dichloromethane (400 mL) slowly over 45 min. The temperature was maintained below 17 °C. Cooling was continued to 6 °C, and the mixture was stirred an additional 40 min. The reaction was monitored by ^{1}H NMR. The mixture was quenched with water (3 L), the layers were separated, and the aqueous layer was extracted with dichloromethane (3 L). The combined organics were washed with a 0.1 N HCl solution (2 L) followed by a saturated solution of sodium bicarbonate (2.0 L). These two aqueous layers were back extracted with dichloromethane (500 mL/each). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to afford **8** as an off-white solid. The yield of **8** was not determined and was carried on to next reaction without purification. ^{1}H NMR of off-white solid 4.60 (bs, 1H), 4.20−4.22 (m, 1H), 4.12−4.16 (m, 1H), 3.95 (bs, 1H), 3.03 (s, 3 H), 1.44 (s, 9H), 1.23 (d, J = 6.9 Hz).

Preparation of (*R*)-(2-Cyano-1-methyl-ethyl)-carbamic Acid *tert*-Butyl Ester (9). Sodium cyanide (625 g, 12.75 mol) was suspended in dimethyl sulfoxide (3.5 L) and warmed to 40 °C. (**CAUTION**: The solution of NaCN in DMSO could be readily absorbed through the skin. Appropriate protective equipment and safe disposal of all waste is required[18]—aqueous streams containing cyanide should be kept alkaline to prevent the release of HCN gas.) A solution of (*R*)-methanesulfonic acid 2-*tert*-butoxycarbonylamino-propyl ester **8** (assuming 90% conversion in previous step, 4.25 mol) in dimethyl sulfoxide (1 L) was added to the suspension over 30 min while warming to 50 °C. **Caution**: an exotherm was observed when the reaction temperature reached 50 °C and 600 mL of mesylate solution was added. The temperature increased to 60 °C before it started to decrease to 55 °C. The remainder of the mesylate solution was added slowly to maintian a temperature of 55 °C. A thick orange suspension formed. The suspension was cooled to 50 °C, stirred for 4 h, then cooled further to 25 °C, and stirred an additional 10 h. The reaction was monitored by ^{1}H NMR. To the orange suspension was added water (4.25 L), and the temperature increased slightly (25.9 to 34.0 °C). The dark-maroon solution was extracted with diethyl ether (4 × 4 L). The combined organic layers were washed with brine (2 × 1.5 L). The resulting brine layers were then back extracted with diethyl ether (4 × 0.5 L). The combined diethyl ether layers were dried (magnesium sulfate) and concentrated in vacuo to afford a dark-brown oil. The brown oil was taken up in warm diethyl ether (500 mL), and hexanes (4 L) was added. The mixture was tumbled and warmed to 50 °C to solubilize the mixture and then cooled slowly to 0 °C. The resulting precipitate **9** (600 g) was collected on a glass frit and dried under high vacuum. The mother liquor was taken up in hexane (1 L) and a minimal amount of diethyl ether and passed through a silica gel plug (600 g), eluting with hexane/ethyl acetate (3:1). The product-containing fractions were concentrated to dryness and subjected to recrystallization from hexanes. The resulting crystals (25 g) were collected by vacuum filtration to afford a total of 625 g of **9**. ^{1}H NMR 4.67 (bs, 1H), 3.93−3.96 (m, 1H), 2.74 (dd, J = 16.5, 4.8 Hz, 1H), 2.52 (dd, J = 16.7, 4.1 Hz, 1H), 1.44 (s, 9H), 1.31 (d, J = 6.9 Hz).

Preparation of (*R*)-3-Amino-butyronitrile (10). To a cooled (9 °C) solution of (*R*)-(2-cyano-1-methyl-ethyl)-carbamic acid *tert*-butyl ester **9** (633 g, 3.44 mol) in tetrahydrofuran (1 L) was added 6 N hydrochloric acid (1.7 L, 10.2 mol). **Caution**: it is critical that no cyanide remain in **9** from the previous step as HCN will form upon acid addition; bubbling occurs, and the reaction is slightly exothermic (9.1 to 18.7 °C). The mixture was cooled to 14 °C, the ice bath was removed, and the reaction was warmed to 22 °C over 40 min. The reaction was warmed to 40 °C over 1 h and stirred for an additional 50 min when an aliquot was taken for ^{1}H NMR. The reaction was cooled to ambient temperature, and the tetrahydrofuran was removed in vacuo. The resulting aqueous mixture was cooled to 10 °C. A 40% sodium hydroxide solution (1.2 L) was added slowly (to pH > 12) at <30 °C. Sodium chloride (300 g) was then added (to increase subsequent extraction efficiency). The aqueous mixture was extracted with a methanol/dichloromethane (1:9) solution

(4 × 4 L). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to afford an orange oil **10** (259.1 g, 90%). The material was used without further purification. ^{1}H NMR 3.22−3.33 (m, 1H), 2.47−2.62 (m, 2H), 1.17 (d, J = 6.3 Hz, 3H).

Preparation of 1-Ethyl-1-methyl-4-oxo-piperidinium Iodide (11). To a solution of 1-methyl-4-piperidone (295 mL, 2.53 mol) in acetone (2.5 L) was added a solution of iodoethane (245 mL, 3.05 mol) in acetone (0.5 L). The mixture was warmed to 60 °C and stirred for 19 h under nitrogen (a white precipitate begins to form in the solution after about 1 h). The solvent was evaporated from the reaction vessel resulting in off-white solid. The solid was resuspended in acetone (4.5 L) with stirring and the suspension was cooled to 0 °C and stirred for 3.5 h. The solid was collected by vacuum filtration, washed with acetone (2 L) and air-dried for 30 min to afford an off-white solid **11** (539.74 g, 79%). ^{1}H NMR 3.44−3.55 (m, 6H), 3.09−3.11 (m, 3H), 2.07 (bs, 4H), 1.36−1.40 (m, 3H).

Preparation of (*R*)-3-(4-Oxo-piperidin-1-yl)-butyronitrile (2). To a warm (40 °C) solution of (*R*)-3-amino-butyronitrile **10** (259.1 g, 3.08 mol) in ethanol (3 L) and water (1.5 L) was added a solution of 1-ethyl-1-methyl-4-oxo-piperidinium iodide **11** (976.67 g, 3.62 mol) in water over 10 min. The mixture was warmed to 67 °C over 2 h and stirred for an additional 2 h. The reaction progress was monitored by ^{1}H NMR. Once complete (typically 2 h), the reaction was cooled to 30 °C, and the ethanol was removed in vacuo. The resulting aqueous layer was diluted with saturated sodium bicarbonate solution (1 L), and the product was extracted with dichloromethane (4 × 4 L). The combined dichloromethane layers were dried (sodium sulfate) and concentrated in vacuo to afford 400 g of crude product. The product **2** was purified by flash chromatography on silica gel (2 kg), eluting with ethyl acetate (12 L) to afford **2** as an orange oil (297 g, 58%). The purity of **2** by gas chromatography (GC) was 99.45%, and the chiral purity by GC was 99.58% ee. ^{1}H NMR 3.20−3.27 (m, 1H), 2.81−2.85 (m, 4H), 2.38−2.58 (m, 6H), 1.22 (d, J = 6.9 Hz, 3H); ^{13}C NMR 208.90, 118.79, 56.36, 48.53, 42.06, 22.33, 15.63.

Preparation of 2-Amino-4,6-dimethyl-5-bromopyridine (12). 2-Amino-4,6-dimethylpyridine (150 g, 1.23 mol) was dissolved in dichloromethane (3.6 L) at RT under a N_2 blanket with mechanical stirring. After all the solid had dissolved, cooling was started. When the solution had cooled to ≤ −5 °C, solid *N*,*N*-dibromo-4,4,-dimethylhyndantoin (DBH, 176 g, 0.5 equiv, 0.62 mol) was added in ∼40-g portions with vigorous stirring. The temperature was allowed to drop below −5 °C before adding another portion: total addition time ∼30 min. The reaction was stirred at for 1 h, maintaining the internal termperature at −5 °C. A reaction aliquot was withdrawn and quenched with saturated Na_2SO_3; the mixture was extracted with DCM; the organic layer was removed and concentrated; the residue was dissolved in $CDCl_3$ and the NMR taken, with the disappearance of 6.13 ppm peak being diagnostic of reaction progress. Additional DBH was added based on the NMR integration of the remaining starting material. After another 1 h, the reaction was quenched with a Na_2SO_3 solution (15 g Na_2SO_3 in 2 L water), and the reaction temperature was increased to 10 °C. NaOH (10 M) was added until the equilibrated pH was 13−14 (∼100 mL used). The organic layer was removed, and the aqueous layer was extracted with DCM (1 L × 2). The combined organic layers were dried with Na_2SO_4 and concentrated on a rotary evaporator. Spontaneous crystallization occurred, and the evaporation was continued to near dryness. The solid was triturated in hexane−ether (1:1, 0.5 L) until no clumps were visible and the solids were isolated by filtration. The filter cake was washed with hexane−DCM (1:1, 0.3 L, slurry), and vacuum-dried to give the product, 2-amino-4,6-dimethyl-5-bromopyridine as white crystals (186 g, 74.4%). The mother liquor was purified by flash chromatography (2 L silica, 1:1→2:3→1:2 hexane−EtOAc as the eluent) to give another crop of product (70 g, ∼14%). Overall yield of **12** was 420 g (85%). ^{1}H NMR 6.25 (s, 1 H), 4.27 (br s, 2 H), 2.50 (s, 3 H), 2.28 (s, 3H).

Preparation of 2-Chloro-4,6-dimethyl-5-bromopyridine (13). Pyridine·HCl (520 g, 3 equiv, 3.34 mol) was suspended in DCM (2 L) at RT. 2-Amino-4,6-dimethyl-5-bromopyridine (**12**, 300 g, 1.49 mol) was added in one portion and was rinsed down with DCM (0.5 L). CuCl (15 g, 0.1 equiv, 0.15 mol) was added, followed by solid $NaNO_2$ (308 g, 3 equiv, 3.34 mol) in one portion. Concentrated HCl (6 mL, 0.04 equiv, 2% v/w vs SM) was added slowly via a pipet, and the mixture was stirred vigorously at RT. After 1.5 h, an aliquot of the reaction mixture was analyzed by NMR (watch for the disappearance of 6.25 ppm signal) and showed a complete and clean reaction. The reaction was carefully quenched with saturated $NaHCO_3$ (1.2 L), and the mixture was stirred for 15 min at RT. The layers were separated, and the aqueous was extracted with DCM (0.6 L × 2). The combined organic layers were dried with Na_2SO_4, and concentrated by rotary evaporation. The residue was diluted with hexane (0.5 L) and equilibrated with HCl (100 mL concentrated HCl in 1.5 L of water, final pH ∼7). The mixture was extracted with ether (0.5 L × 4), and the combined extract was washed with brine (0.5 L). The organic layer was dried with Na_2SO_4 and filtered through a silica plug (1 L silica on a 2.5 L glass frit). The silica plug was washed with ether (1 L), and the filtrate was concentrated to dryness. The residue was triturated in hexane (400 mL), filtered, and washed with hexane (4−500 mL). The solid was dried under high vacuum to give 2-chloro-4,6-dimethyl-5-bromopyridine (**13**) as off-white crystals (123 g, 38%). The mother liquor was concentrated and resubjected to silica plug/crystallization to give another crop of product (53 g, 16%). ^{1}H NMR 7.05 (s, 1 H), 2.65 (s, 3 H), 2.39 (s, 3 H).

Preparation of 6-Chloro-2,4-dimethylnicotinic Acid (5). LiCl (anhydrous, 39.81 g, 1.2 equiv, 0.94 mol) was placed in a flask equipped with mechanical stirring, followed by THF (0.5 L) and *i*-PrMgCl (2 M in THF, 470 mL, 1.2 equiv, 0.94 mol, added through a dropping funnel over 4 min). The mixture was stirred at RT until LiCl had all dissolved. Solid 2-chloro-4,6-dimethyl-5-bromopyridine (**13**, 172 g, 0.78 mol) was added under a strong N_2 flow over 2 min. The mixture was stirred for 2.5 h, after which an aliquot NMR confirmed a complete reaction: Approximately 20 μL of reaction mixture was withdrawn with a gastight syringe, quenched with MeOH-d_4 in a NMR tube, and used directly. SM: ^{1}H NMR (MeOH-d_4) 7.25 (s, 1 H), 2.60 (s, 3 H), 2.41 (s, 1 H); for exchanged product: δ 7.11 (s, 1H, 2.44 (s, 3 H), 2.33 (s, 3 H); for proteo-debrominated material (if any): 7.11 (s, 1H), 7.08 (s, 1 H), 2.44 (s, 3 H), 2.33 (s, 3 H). The mixture was cooled in an ice/water bath, and CO_2 was introduced (after the internal temperature had dropped below 10 °C) with vigorous stirring. After 40 min, the reaction mixture was concentrated until foaming started. The residue was cooled to ∼30 °C, and water (175 mL, 1 wt equiv vs SM) was added. Concentrated HCl (∼85 mL, 1.2 equiv, 0.93 mol) was added dropwise under vigorous stirring. DCM (60 mL) was added, and rapid stirring was continued for 0.5 h, during which time crystallization took

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place. The solid was filtered, placed in a crystallizing dish, and further dried in a vacuum oven at 60 °C for 2 d to give 6-chloro-2,4-dimethylnicotinic acid (125.57 g, 87%). The mother liquor was adjusted to pH 2 with concentrated HCl to give more solid, which was filtered, dried, and then dissolved in MeOH (20 mL) and diluted with DCM (40 mL). Hexane (200 mL) was added, and the mixture was heated until all solids had dissolved. The solution was cooled slowly to RT and further held in an ice/water bath for 0.5 h. The crystals were filtered and dried in a vacuum oven overnight to give another crop of product (9.31 g, 6%). ^{1}H NMR (MeOH-d_4) 7.23 (s, 1 H), 2.51 (s, 3 H), 2.37 (s, 3 H). 94.8% chemical purity by GC.

Preparation of 3-(Aminomethyl)-thiophene (3). A solution of lithium aluminum hydride in THF (1.0 M, 3.7 L, 3.7 mol) was added to 0 °C THF (7 L). A solution of 3-cyanothiophene (402 g, 3.68 mol) in THF (600 mL) was then added over 20 min, at <20 °C. A rinse of the dropping funnel with ~50 mL of THF was used to ensure all the nitrile was transferred. The reaction was warmed to 50 °C over 2 h, and was maintained at that temperature until the reaction was complete by ^{1}H NMR: ~2 h. The reaction mixture was cooled to 10−15 °C and was quenched through slow addition of the following reagents, in sequence (at <30 °C: water (140 mL), 4 N NaOH (210 mL), and water (420 mL). The fine white suspension of aluminum salts was removed by filtration through a glass frit, and the filter cake was washed with diethyl ether (4 × 1 L). The combined organic fractions were then concentrated by rotary evaporation, and the oily residue was purified by fractional distillation under vacuum (product collected at 95−105 °C at 25−30 mmHg vacuum) to give 355 g (85%) of **3** as a colourless oil. ^{1}H NMR 7.31 (m, 1H), 7.15 (m, 1H), 7.03 (m, 1H), 3.68 (s, 1H).

Preparation of *N*-Methoxy-(4-nitrophenyl)-carbamate (14). A solution of methoxylamine hydrochloride (137 g, 1.64 mol) in water (3.0 L) was cooled to 0−2 °C. Sodium bicarbonate (230 g, 2.73 mol) was added, and dichloromethane (1.8 L) was added. A solution of 4-nitrophenyl chloroformate (300 g, 1.49 mol) in dichloromethane (1.2 L) was then added over 20 min, at <10 °C. A white precipitate formed during the chloroformate addition. The reaction mixture was then cooled to 0 °C, and was stirred for 20 min. The solids were collected by filtration, and the filter cake was washed with water (1 L). The solids were dried under vacuum (no heating) to give **14** (262 g, 88%). ^{1}H NMR 8.27 (d, 2H, J = 9.1 Hz), 7.83 (br s, 1H), 7.36 (d, 2H, J = 9.1 Hz), 3.85 (s, 3H).

Preparation of (*R*)-3-[4-(Thiophen-3-ylamino)-piperidin-1-yl]-butyronitrile (15). 3-(Aminomethyl)-thiophene (274 g, 2.42 mol) was added to dichloromethane (10 L). The solution was cooled to 8−10 °C, and then acetic acid (139 mL, 4.84 mol) was added (temperature rose to 14 °C). Sodium triacetoxyborohydride (770 g, 3.63 mol) was added, rinsing forward with 100 mL of dichloromethane. The reaction mixture was cooled to 8−10 °C, then (*R*)-3-(4-oxo-piperidin-1-yl)-butyronitrile (**6**, 422 g, 2.53 mol) in dichloromethane (600 mL) was added over 20 min, at <20 °C. The reaction was stirred at 20−22 °C until complete by ^{1}H NMR (~2 h). Water (5 L) was added over 5 min (effervescence), and then conc. HCl (465 mL) was added (target pH of aqueous layer was 4.0). The organic and aqueous layers were separated, and the aqueous layer was washed with dichloromethane (2 L). To the aqueous layer was added 10 M sodium hydroxide (1300 mL) to achieve a target pH of 8. The aqueous layer was then extracted with dichloromethane (6 × 2.5 L). The combined organic fractions were washed with a solution of 10 M NaOH and saturated sodium chloride (10: 1 ratio, 2 L total). The organic fraction was dried with sodium sulfate, filtered, and concentrated. The residue was taken up in a 1:1 mixture of methyl *tert*-butyl ether and hexane (400 mL total) and was warmed slightly to solubilize. The solution/suspension was cooled to 4 °C overnight. The product **15** was isolated by filtration, and the crystals were washed with a mixture of hexanes and MTBE (3:2 ratio, 3 × 400 mL) to give 380 g as a first crop. The washings and mother liquor were concentrated and taken up in MTBE and hexanes (1:1 mixture, 500 mL), warming to 30 °C to solubilize. The solution was cooled to 0−4 °C overnight to give a second crop of crystals (140 g). Total yield of **15**: 520 g (82%). If desired, the filtrate can be subjected to a repeat of the concentration/recrystallization cycle to obtain slightly more material (8−10% increase in yield). ^{1}H NMR 7.29 (m, 1H), 7.12 (s, 1H), 7.04 (d, 1H, J = 4.9 Hz), 3.83 (s, 2H), 3.04 (m, 1H), 2.83 (m, 2H), 2.54 (m, 2H), 2.20−2.38 (m, 3H), 1.90 (m, 2H), 1.35 (dq, 2H, J = 11.2, 3.4 Hz), 1.19 (d, 3H, J = 6.7 Hz). Purity of combined batches >99% by HPLC.

Preparation of (*R*)-3-[4-(Thiophen-3-ylamino)-piperidin-1-yl]-butyl-1-amine (4). To a nitrogen-purged reactor (equipped with overhead stirring, a dropping funnel and condensor) was added lithium aluminum hydride (116.1 g, 3.05 mol), followed by slow addition of MTBE (6 L). The mixture was warmed to 45 °C, and then a solution of nitrile **15** (536.5 g, 2.04 mol) in MTBE (0.5 L) was added slowly over 45 min, at <52 °C. The mixture was stirred a further 60 min—at which point an aliquot examined by NMR determined that the reaction was complete. The mixture was cooled over 60 min to 30 °C and was quenched via a sequential addition of the following: water (116 mL), 4 N NaOH (174 mL), water (350 mL) at <50 °C. The suspension was cooled to 30 °C and then was filtered, washing the aluminum salts with MTBE (4 × 800 mL). The filtrate was concentrated to give **4** as a colourless oil (538 g, 87%) which was used directly in the next reaction. ^{1}H NMR 7.25 (dd, 1H, J = 4.9, 4.0 Hz), 7.11 (m, 1H), 7.02 (dd, 1H, J = 4.9, 0.8 Hz), 3.82 (s, 2H), 7.73 (m, 4H), 2.48 (m, 1H), 2.35 (dt, 1H, J = 11.2, 2.3 Hz), 2.11 (dt, 1H, J = 11.2, 2.3 Hz), 1.88 (m, 2H), 1.63 (hex, 1H, J = 7.1 Hz), 1.36 (m, 6H), 0.94 (d, 3H, J = 6.6 Hz).

Preparation of 6-Chloro-*N*-{(*R*)-3-[4-(thiophen-3-yl)-piperidin-1-yl]-butyl}-2,4-dimethylnicotinamide (6). To a solution of amine **4** (538 g, 2.01 mol) in dichloromethane (12 L) was added carboxylic acid **2** (381 g, 2.01 mol). HOBt (285 g, 2.11 mol) and EDCI (404 g, 2.11 mol) were then added, rinsing forward with 0.5 L dichloromethane. (**Caution**: HOBt has been reported to have explosive properties, especially in anhydrous forms. The reagent should contain >10% water by mass to be considered desensitized[19].) The mixture was stirred under nitrogen at ambient temperature for 16 h. Water (7 L) was then added, and while the mixture was stirred, the pH of the aqueous layer was adjusted to 6 with concentrated HCl (~200 mL). The layers were then separated, the organic layer was washed with water (5 L), and the combined aqueous layers were washed with dichoromethane (3 L). The aqueous layer (containing product) was then adjusted to pH 10 with 10 M NaOH solution (380 mL), and the mixture was extracted with dichloromethane (3 × 3 L), adjusting the pH of the aqueous layer between extractions. The combined dichloromethane fractions were concentrated to ~1.5 L volume and were filtered through silica gel (2 kg) which had been preconditioned with 10% methanol in dichloromethane. The product was eluted with 1% NH_4OH, 10% methanol in dichloromethane (monitored by TLC). Fractions containing

product were concentrated. To the viscous oil was added MTBE (1.5 L), and the mixture was warmed to 30−35 °C with vigorous stirring. A crystalline suspension began to form, and then hexanes (1 L) was added. The suspension was stirred for another 20 min and then was allowed to stand for 30 min before being filtered. The product **6** was collected as a pale-yellow, crystalline solid (528 g, 61%). It should be noted that the mother liquor can be harvested, rechromatographed, and recrystallized to provide a further 10−20% yield, if desired. ^{1}H NMR 8.77 (m, 1H), 7.08 (m, 1H), 7.02 (m, 2H), 3.86 (m, 1H), 3.70 (s, 2H), 3.33 (m, 1H), 2.83 (m, 2H), 2.44 (m, 1H), 2.51 (s, 3H), 2.37 (dt, 1H, J = 9.8, 1.8 Hz), 2.33 (m, 1H), 2.30 (s, 3H), 1.73 (m, 3H), 1.53 (m, 1H), 0.94 (d, 3H, J = 6.6 Hz), 0.74 (m, 3H). Purity 99.1% by HPLC.

Preparation of 6-Chloro-*N*-{(*R*)-3-[4-(3-methoxy-1-thiophen-3-ylmethyl-ureido)-piperidin-1-yl]-butyl}-2,4-dimethylnicotinamide (1). Dichloromethane (2 L) was added to a reactor, and the solvent was cooled to 15 °C. Amine **6** (500 g, 1.15 mol) was then added, rinsing forward with dichloromethane (1 L). Carbamate reagent **14** (256 g, 1.21 mol) was then added, again rinsing forward with dichloromethane (0.5 L). The mixture formed a pale-yellow suspension. Diisopropylethylamine (240 mL, 1.37 mol) was added over 5 min, rinsing forward with dichloromethane (0.5 L). At the completion of addition of the DIPEA, all the solids dissolved. After 60 min, the reaction was complete by ^{1}H NMR aliquot. The reaction was quenched with water (4 L), and the pH of the aqueous layer was adjusted to 1−2 (target 1.5) with slow addition of concentrated HCl (225 mL). Diethyl ether (8 L) was added, and the organic and aqueous layers were separated. The aqueous layer was washed with diethyl ether (3 × 4 L) to remove the yellow *p*-nitrophenol. Dichloromethane (3 L) was added to the aqueous layer, and the pH adjusted to 7 with 4 M NaOH solution (270 mL). Saturated aqueous sodium bicarbonate (1 L) was added, and the pH adjusted to 8 with 4 M NaOH (100 mL). The aqueous and organic layers were separated, and the aqueous layer washed with dichloromethane (3 L). The combined organic layers were dried with sodium sulfate and were concentrated. The residue was reconstituted in dichloromethane (50 mL) and was filtered through silica gel (1 kg), which had been preconditioned with 1% NH_4OH, 5% MeOH in ethyl acetate. The filtration was monitored by TLC, eluting first with 1% NH_4OH, 5% MeOH in ethyl acetate, and then 25% MeOH, 10% NH_4OH in ethyl acetate, and was intended to remove high-polarity baseline residual reagents and impurities. The fractions containing product were then concentrated, taken up in methanol, and concentrated again (to remove ethyl acetate). The product was dried under vacuum in a 40 °C oven for 48 h to produce an amorphous light-yellow, foamy solid (**1**) in a yield of 563 g (96%). ^{1}H NMR ($CDCl_3$) δ 8.76 (d, 1H, J = 5.5 Hz), 7.38 (dd, 1H, J = 5.0, 3.0 Hz), 7.12 (m, 1H), 7.03 (m, 2H), 6.93 (m, 1H), 4.22 (m, 1H) 3.84 (m, 1H), 3.68 (m, 2H), 3.66 (s, 3H), 3.26 (m, 1H), 2.83 (m, 2H), 2.72 (m, 1H), 2.57 (t, 1H, J = 11.4 Hz), 2.53 (s, 3H), 2.26 (m, 3H), 2.16 (t, 1H, J = 11.4 Hz), 1.76−1.66 (m, 3H). 1/54 (m, 1H), 1.02 (m, 1H), 0.98 (d, 3H, J = 6.6 Hz), 0.88 (m, 1H). ^{13}C NMR 167.4, 159.4, 155.7, 150.4, 147.9, 139.1, 133.2, 127.9, 126.4, 122.8, 121.7, 64.6, 60.9, 52.6, 52,1, 43.6, 41.2, 40.5, 31.0, 30.0. 22.4, 19.1, 13.8. MS m/z 508 (m + H). HPLC purity 99.84%. Chiral HPLC purity >99.5% ee. Anal. Calcd For $C_{24}H_{34}N_5ClO_3S$: C, 56.74; H, 6.74; N, 13.78; Cl, 6.98; S, 6.31. Found: C, 56.94; H, 6.82; N, 13.76; Cl, 7.14; S, 6.23.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. NMR spectra for **1**, **4**, **6**, **14** and **15** are provided. This information is available free of charge via the Internet at http://pubs.acs.org.

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